region was amplified in this experiment based on an assumption that the polymorphic nature of this region might help the clear distinction of each mycobacterial species using molecular biological techniques such as PRA and PCR-DNA hybridization. On the other hand, the region of the *rpoB* gene was also chosen to be flanked by highly conserved sequences, thus can be suitable for PCR amplification of the *rpoB* region of all mycobacterial species using the same set of PCR primers.

Replace the first full paragraph at page 13 with the following:

PCR was carried out in a final volume of 50 µl with 10 µl of DNA supernatant containing approximately 10 ng of genomic DNA, 10 pmole of each primer, 2 mM $MgCl_2$, 200 μM of deoxynucleotide triphosphates, and 1 unit of DYNAZYME® II DNA polymerase (FINNZYMES, Espoo, Finland). DNA samples were first denatured completely by incubation at 94°C for 5 min before amplification cycle, then amplified using a cycle that includes (1) denaturation at 94°C for 1 min, (2) primer annealing at 58°C for 1 min, and (3) elongation at 72°C for 1 min for 35 times using a thermocycler (model 9600, Perkin Elmer). After the last amplification cycle, the samples were incubated further at 72°C for 7 min for complete elongation of the final PCR products. Positive and negative controls were always included in each PCR reaction. The positive control was the PCR mix with DNA of reference strain, *M. bovis,* and the negative control was the PCR mix without any DNA. After the PCR, the amplification results were visualized using 1.5% agarose gel electrophoresis and ethidium bromide staining.

Replace the paragraph spanning pages 14-15 with the following:

Cloning and sequence analysis. For sequence analysis, PCR products were purified by using a GENECLEAN® kit (BIO101, Vista, Calif. USA) from an agarose gel and cloned into TOPO-TA cloning vector (Invitrogen Co., Carlsbad, CA) by the method recommended by the manufacturer. DNA sequencing was done by the dideoxy nucleotide-chain termination method (21) using ARL automatic sequence (Pharmacia Biotechs, Uppsala, Sweden). For each clone, M13 reverse primer and T7 promoter

primer were used separately to read sequences from both directions. Sequences were aligned using a multiple sequence alignment program (6).

Replace Table 2 spanning pages 15 and 16 with the following:

Table 2. Oligonucleotide probes designed in this study to develop PCR-probe hybridization assay for Mycobacterial species identification.

Name of oligonucleotides	Sequences of oligonucleotides	Target Mycobacteria
PAN-MYC	GACGTCGTCGCCACCATCGA (nucleotides 108 to 127 of SEQ ID NO:1)	All mycobacterial species
TB	CATGTCGGCGAGCCC (nucleotides 66 to 80 of SEQ ID NO:5)	*M. tuberculosis* complex
AVIUM	CGGTGAGCCGATCACCA (nucleotides 71 to 87 of SEQ ID NO:15)	*M. avium*
INTRA	CCTGCACGCGGGCGA (nucleotides 62 to 76 of SEQ ID NO:20)	*M. intracellularae*
GORDONAE	GTCGGCGATCCGATCA (nucleotides 69 to 84 of SEQ ID NO:1)	*M. gordonae*
SZULGAI	TCTGAACGTCGGCGAG (nucleotides 61 to 76 of SEQ ID NO:12)	*M. szulgai*
KANSASII	GGCCACGATGACCGTG (nucleotides 155 to 170 of SEQ ID NO:8)	*M. kansasii*
GASTRI	TCTGAACGTCGGCGAG (nucleotides 61 to 76 of SEQ ID NO:12)	*M. gastri*
FORTUITUM	CCTGAACGCCGGCCAG (nucleotides 62 to 77 of SEQ ID NO:19)	*M. fortuitum* *M. fortuitum complex*
SCROFULACEUM	CGTACGGATGGCCAGC (nucleotides 153 to 168 of SEQ ID NO:9)	*M. scrofulaceum*
CHELONAE	TGGTGACTGCCACCACG (nucleotides 85 to 101 of SEQ ID NO:7)	*M. chelonae*
ABSCESSUS	AGGTGACCACCACCACC (nucleotides 85 to 101 of SEQ ID NO:21)	*M. abscesus* *M. terrae*

Name f ligonucle tid s	Sequences f ligonucleotid s	Target Mycobacteria
ULCERANS/ MARINUM	GGCCAGCCCATCACC (nucleotides 72 to 86 of SEQ ID NO:10)	*M. ulcerans / M. marinum* *M. genavanse / M. simiae*

Replace the paragraph spanning pages 16-17 with the following:

PCR-reverse blot hybridization. All oligonucleotide probes to be applied on the membrane were synthesized with 5' terminal amino group, which link the oligonucleotides to the BIODYNE® C membrane (Pall BioSupport, East Hills, NY) by forming covalent bond with negatively charged carboxyl group fixed on the membrane. Before blotting the oligonucleotide probes, the BIODYNE® C membrane was activated by incubating in 10 ml of freshly prepared 16% (w/v) 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDAC). After rinsed with the water, the membrane was placed on a support cushion in a clean miniblotter system (Immunetics, Inc., Cambridge, MA), and the residual water was removed from the slots. Then, the slots were filled with 150 μl of the diluted oligonucleotide solutions (approximately 200 pmol to 1 nmol of oligonucleotides in 150 μl of 50 mM $NaHCO_3$, pH 8.4). Subsequently, the membrane was incubated for 1 hr at room temperature, and then excess amount of oligonucleotide solution was removed from the slots by aspiration. In order to inactivate the membrane, the membrane was removed form the miniblotter using forceps, incubated in 100 mM NaOH for 10 min in a rolling bottle, and washed in 100 ml 2x SSPE/0.1% SDS for 5 min at 60°C in a plastic container under gentle shaking. Before applying PCR products on the BIODYNE® C membrane, the membrane was incubated for 5 min at room temperature in 100 ml 2x SSPE/0.1% SDS.

IN THE CLAIMS

Kindly enter the claims.